

# METHODS IN MOLECULAR BIOLOGY™

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# **Neuropeptides**

## **Methods and Protocols**

Edited by

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## Preface

The term *neuropeptide* was originally coined to indicate small protein molecules that are contained in neurons. In the late 1970s and the 1980s of the last century, several tens of neuropeptides were localized by immunocytochemistry to discrete cell populations of the central and peripheral nervous system, and the concept of *chemical neuroanatomy*, originally developed by Tomas Hökfelt and coworkers, entered the scene of neurobiology. Since then, the field of neuropeptide biology has dramatically widened, and today the ultimate frontiers in neuropeptide research lie in the development of pharmacologically active compounds that are capable of crossing the blood–brain barrier to exert their biological role(s) *in vivo* and in the construction of genetic vectors to be employed in gene therapy.

This book represents a readily reproducible collection of established and emerging techniques for neuropeptide research. Such a collection is preceded by a general introductory chapter (Chapter 1) that discusses a series of new concepts leading to a broader neuropeptide definition in light of the huge amount of data accumulated after more than half a century of neuropeptide research.

The methods presented include immunocytochemical localization, biochemical characterization, functional analysis, development and production of genetic probes, and the design of neuropeptide derivatives for cellular neurobiology as well as the potential therapeutic applications.

As a general indication to the readers, Chapters 2–10 are focused on a series of techniques for localization studies. They cover a broad range of protocols, such as the immunocytochemical detection of neuropeptides in nonmammalian vertebrates together with a detailed description of procedures for anesthesia and tissue preparations in these species (Chapter 2); the combined neuropeptide/receptor localization at the light and transmission electron microscope for connectivity studies (Chapter 3); the analysis of neuropeptide genes' transcription by localization of pre-mRNA (Chapter 6) or mRNA/microRNA with *in situ* hybridization (Chapter 4), *in situ* PCR (Chapter 5), and laser capture/microdissection (Chapter 7); the visualization *in vivo* of neuropeptide secretion (Chapter 8) and translocation across the plasma membrane (Chapter 9); and the functional analysis of neuropeptide interactions *in vitro* with cells of the immune system (Chapter 10).

Chapter 11 describes a series of electrophysiological protocols for functional studies *in vitro* and *in vivo*.

Chapters 12–19 are devoted to biochemical/molecular biology techniques, ranging from radioimmunoassay (Chapter 12) to neuropeptidomics employing reverse-phase HPLC (Chapter 13) or mass spectrometry (Chapter 14), RNA analysis by suppression subtractive hybridization (Chapter 15), determination of neuropeptide release *in vivo* by microdialysis (Chapter 16) or antibody microprobes (Chapter 17), and measurement of neuropeptidases (Chapter 18) and neuropeptide autoantibody levels (Chapter 19) in biological fluids.

Chapters 20–24 deal with a number of techniques developed to optimize neuropeptide administration to central neurons or to interfere with biological effects *in vivo*. These procedures include the intranasal delivery of neuropeptides (Chapter 20), the development of

neuropeptide pro-drugs (Chapter 21), the use of phosphorothioate oligodeoxynucleotides that are capable of crossing the blood–brain barrier to knock down neuropeptides in the CNS (Chapter 22), the development of liposome-encapsulated neuropeptides for assessing the chronic actions of physiologically short-lived molecules (Chapter 23), the construction of recombinant adeno-associated viral vectors that can be used to locally or systemically enhance or silence neuropeptide gene expression (Chapter 24).

Finally, Chapter 25 describes a calcium mobilization assay in mammalian cells to identify novel G-protein-coupled receptor family members that transduce the neuropeptide signals.

All scientists who have excellently contributed to this book have a direct experience in one or more fields of neuropeptide research. I am very much indebted to all of them for their successful effort in emphasizing the description of the more common pitfalls in the techniques that they have described and of the hints to reduce the possibility of failure for beginners.

The collection of protocols that forms this book is surely not exhaustive of the wide range of approaches that today can be employed in top level neuropeptide research. Yet it is intended for a large audience of scientists, including histologists, biochemists, cellular and molecular biologists, and electrophysiologists that are currently active in the field or are willing to enter such an exciting and still expanding area of neurobiology.

*Grugliasco, TO, Italy*

*Adalberto Merighi*

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Antibodies are glycoproteins produced by plasma cells that function to bind foreign or non-self molecules. In response to a foreign antigen, the host can produce a diverse array of antibodies that are structurally similar yet unique in their properties. Neuropeptides: Methods and Protocols presents a readily reproducible collection of established and emerging techniques for neuropeptide research as contributed by expert researchers in the field. The detailed methods presented cover subjects such as immunocytochemical localization, biochemical characterization, functional analysis, development and production of genetic probes, and the design of neuropeptide derivatives for cellular neurobiology as well as the potential therapeutic applications. Written in the highly successful Methods in Molecular Biology series format, chapters include intro